

## Characterization of Hydroxyapatite Doped with Different Concentrations of Magnesium Ions

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**Abstract:** Hydroxyapatite (HA) is a major component and an essential ingredient of normal bone and teeth. It makes up bone mineral and the matrix of teeth and gives bones and teeth their rigidity. It has been used as adsorbents for heavy metals, supports and as catalysts in oxidation reactions. The present research aimed to study the influence of Mg in the growth of the hydroxyapatite crystal and improve the chemical and physical properties of HA due to the doping with Mg ions. HA pure (S1) and Mg loaded hydroxyapatite (S2, S3 and S4) of different magnesium concentrations have been prepared to select the best Mg concentration to use in medical applications. The synthesized samples were characterized by The XRD (X-ray diffraction), FTIR (Fourier transform infrared radiation) spectroscopy, SEM (Scanning Electron Microscope) and EDX (Energy Dispersive X-ray Analyses). The obtained results were found that all the samples have the same crystalline structure. The crystallinity of the samples decreased as the Mg ions concentration increase. SEM show that the Mg ions incorporated in the HA crystal, this indicate that the HA crystal acts as carrier for the Mg ions.

**Keywords:** Hydroxyapatite, stoichiometry, magnesium

### 1. INTRODUCTION

Ceramics are historically the oldest of the synthetic materials and their medical use can be traced back to the Egyptians. Among different classes of bioceramics is hydroxyapatite (Hap)  $[\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2]$  which is one of the most biocompatible material, which has close similarities with inorganic mineral component of bone and teeth [1]. The biological behavior of HA ceramics depends on many factors, in particular, on their chemical and phase composition, microstructure, pore size, and pore volume.

However, the chemical composition of HA is very sensitive to various preparation conditions, so that it is rather hard to regulate its non stoichiometry reproducibly. It is well-known that calcium deficient hydroxyl apatite (CDHA) has higher chemical activity than pure HA and forms biodegradable tricalcium phosphate ( $\text{Ca}_3(\text{PO}_4)_2$  TCP) or TCP/HA by thermal decomposition. In order to enhance the properties of the hydroxyl apatite, Mg ions doped with different concentrations into HA crystal.

Magnesium is known to be an important trace element in bone and teeth. Indeed, despite its low concentration (generally between ~0.5 and 1.5 wt%), it plays a key role in bone metabolism, in particular during the early stages of osteogenesis where it stimulates osteoblast proliferation [2], and its depletion causes bone fragility and bone loss [3]. Furthermore, relationships have been suggested between the magnesium content in enamel and the development of dental caries [4]. HAP can be prepared by different routes like chemical precipitation Solid state method, Hydrothermal method and sol-gel method. Hydroxyapatite (HA) and magnesium (Mg) doped HA nanopowders were successfully synthesized through dry mechanochemical method as it has been confirmed by XRD and FTIR analyses. Magnesium ion ( $\text{Mg}^{+2}$ ) one of the main substitutes for calcium in biological apatites where it is the fourth most abundant cation in the human body (0.44-1.23 wt %) and bone contains 0.72 wt % of  $\text{Mg}^{+2}$  [5]. Thus,  $\text{Mg}^{+2}$  is necessitates in all stages of skeletal metabolism and facilitates in the growth of osteoblast and osteoclastic activities by inhibiting the fragility of bone [6].

Magnesium-substituted HA (Mg-HA) is commonly prepared by precipitation and hydrolysis methods. The maximal amount of  $\text{Mg}^{+2}$  substituting for  $\text{Ca}^{+2}$  varies in previous research reports.

Some studies showed that the maximal amount of Mg substitution is less than 1 wt.% in apatite [7,8]. HA nano particles have been investigated by using X-ray diffraction, X-ray fluorescence, field emission scanning electron microscopy. They found that Mg substitution affects the crystallinity, morphology, crystallite size, structure and thermal stability of HA. crystallites become smaller, more

irregular and form agglomerates. Mg substitution decreases the crystallinity of apatite and the lattice parameters  $a$  and  $c$  decrease with increased Mg substitution. Many research teams have worked on the preparation of apatite and calcium-phosphate implant materials containing low levels of Mg, which has been shown to improve their bioactivity [9,10].

The present work is aimed to prepare hydroxyapatite and dope it by different concentrations of Mg, improving the chemical and physical properties of the biomaterials especially hydroxyl apatite and study the influence of Mg ions on the growth of hydroxyapatite crystal in order to use in the medical applications. Hydroxyapatite doped by different concentrations of Mg to be calcium deficient hydroxyl apatite to improve the hydroxyapatite properties.

## 2. MATERIALS AND METHODS

A wet precipitated method used to prepare calcium phosphate bioceramic (S1) and the other samples are calcium phosphate bioceramic doped by magnesium nitrate of different concentrations (S2, S3, and S4) of [0.004, 0.01, 0.02 mole] of  $Mg(NO_3)_2 \cdot 6H_2O$  respectively

### 2.1. Calcium Phosphate Bioceramic Samples

Calcium phosphate bioceramic powder was prepared by an aqueous precipitation technique using 0.16 Mole of calcium chloride dihydrate [ $CaCl_2 \cdot 2H_2O$ ] as a source of calcium which dissolved in one liter of de-ionized water and 0.096 Mole of di-ammonium hydrogen ortho-phosphate [ $(NH_4)_2HPO_4$ ] as a source of phosphorous which dissolved in one liter of de-ionized water. For preparing the Mg loaded HA samples, [0.004, 0.01, 0.02 mole] of  $Mg(NO_3)_2 \cdot 6H_2O$  dissolved with  $CaCl_2 \cdot 2H_2O$  in one liter of de-ionized water. The pH of both solutions was adjusted to about 10 by the addition of a dilute  $NH_4OH$  solution proper strength. Following pH adjustment, 0.096 Mole [ $(NH_4)_2HPO_4$ ] solution was fed from a burette, in a dropwise manner (2 - 5 ml/min), into a stirred solution of 0.16 M [ $CaCl_2 \cdot 2H_2O$ ] heated to  $60^\circ C$  on a hot plate, during this reaction pH must maintain constant at 10 by the addition of a dilute  $NH_4OH$  solution or  $HNO_3$ . After mixing, the solution was aged and vigorously stirred at its boiling point for about 2 h in a sealed container. The HA precipitates were recovered from the solution by sucking filtration and washed three times with deionized water, followed by drying at  $100^\circ C$  overnight. The powder was dis-agglomerated by gently grinding.

## 3. CHARACTERIZATION

X-ray diffractometer ((D8 ADVANCE, BRUKER, AxS Germany) was used to evaluate the phase transformation and crystallite size of the precursor powder calcium-phosphate bioceramics without sintering (as-dry) using Cu  $K\alpha$  radiation ( $\lambda=1.5405 \text{ \AA}$ ) with scanning rate  $0.1^\circ$  in the  $2\theta$  ranging from  $10^\circ$  to  $80^\circ$  step time 1 sec. The average crystallite size of the samples is calculated using the Scherrer formula [11].

$$L_{hkl} = \frac{K\lambda}{\beta \cos \theta_{hkl}}$$

$\beta$  is the full width of the peak at half of maximum intensity (rad) (FWHM),  $\lambda$  is the wavelength of monochromatic X-ray beam radiation. The lattice parameters ( $a$  and  $c$ ) were determined through the (hkl) peaks positions of apatites from XRD patterns according to the following formula [12,13]:

$$1/d^2 = 4/3 \left( \frac{h^2 + hk + k^2}{a^2} \right) + l^2/c^2$$

and the unit cell volume:

$$V = \frac{\sqrt{3}}{2} a^2 \cdot c$$

Fourier transform infrared spectroscopy (FTIR) (Model 1600, Perkin-Elmer) was used to determine the surface functional groups of the calcium phosphate. The FTIR spectrum was recorded in a spectral range of  $4000-400 \text{ cm}^{-1}$ . Each sample used for infrared spectroscopic analysis was prepared according to standard procedure by mixing about 2.00 mg of powder sample with 200 mg of KBr, which was subsequently pressed into pellet in an evacuated die. Microstructure observation for calcium phosphate samples was performed using scanning electron microscope (SEM). Using SEM Quanta 250 FEG (Field Emission Gun) attached with EDX Unit (Energy Dispersive X-ray Analyses), with accelerating voltage 30 K.V., magnification 14x up to 1000000 and resolution for Gun. 1n).

#### 4. RESULTS AND DISCUSSION

The XRD analysis of the as prepared calcium-phosphate bioceramic powder, revealed that there is no secondary phases besides the apatitic phase. The XRD patterns of prepared samples are similar to that of pure calcium-hydroxyapatite JCPDS card No. (9 - 432). There is no peak of crystalline magnesium was detected in the diffractograms as shown in fig.(1).

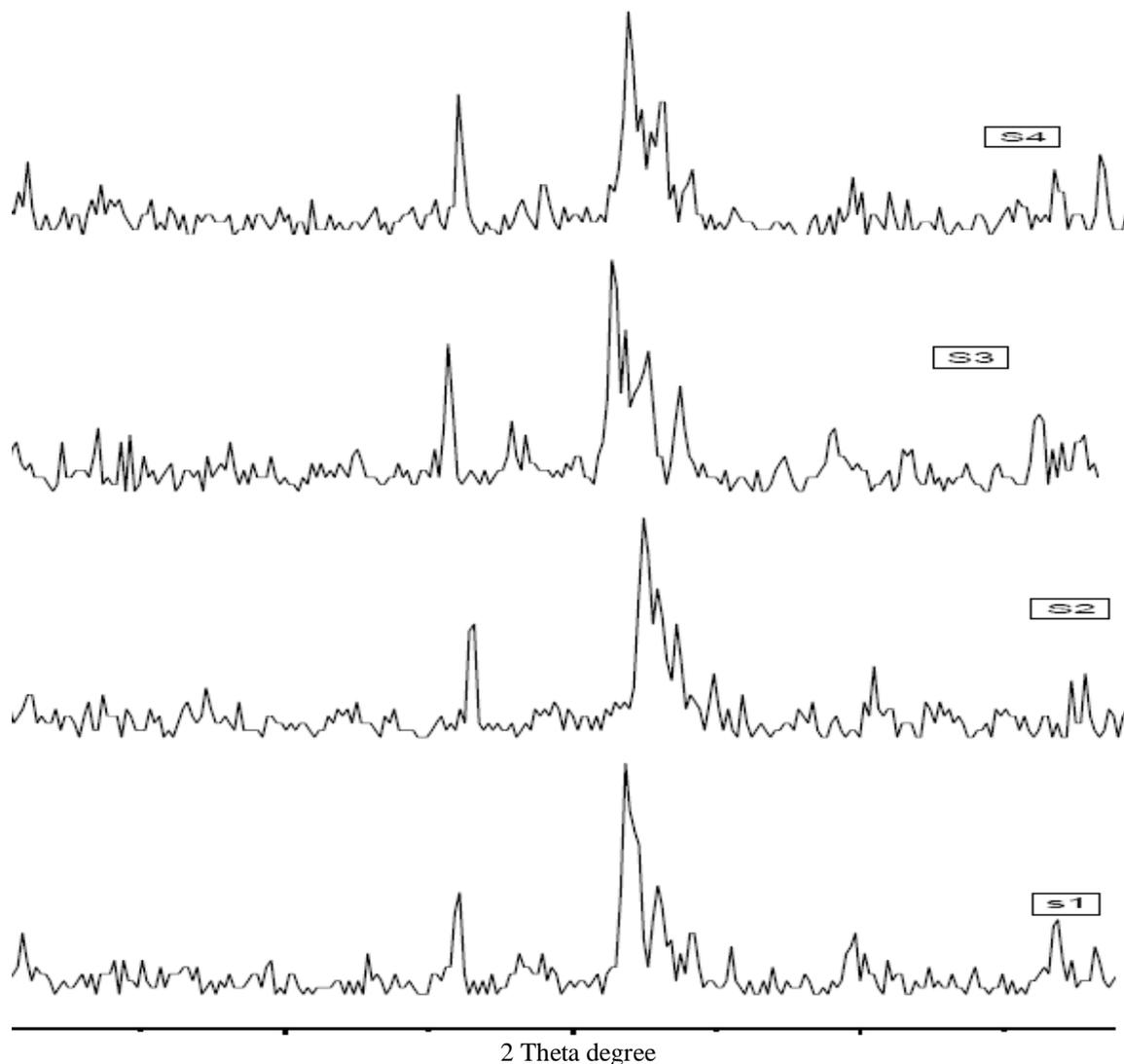
The obtained values of unit cell parameters and unit cell volumes are listed in table (1), which is confirmed with other researchers [14,15]. The lattice parameters of CaHAp and Mg/CaHAp shows that both (a) and (c) parameters decrease upon magnesium addition at S2. Also the volume of the lattice undergoes a contraction. This behavior is due to the fact that The ionic radii of  $Mg^{+2}$  (0.66 Å) is smaller than the radius of  $Ca^{+2}$  (0.99 Å) [16].

**Table1.** Lattice parameters and unit cell volume of the prepared samples (S1, S2, S3 and S4).

	Average of lattice parameter (a) a=b	Average of lattice parameter (c)	Unit cell volume(A <sup>3</sup> )
S1	9.37	6.91	525
S2	9.23	6.85	505.4
S3	9.27	6.86	510.5
S4	9.38	6.86	518

Then it observed that the lattice parameter and the volume began to increase again at S3 and S4, and this is may be due to the crowded of the Mg ions in the HA crystal.

Intensity



**Fig1.** The XRD pattern of prepared HA(S1) and Mg loaded hydroxyl apatite (S2,S3&S4) of concentrations [0.004 ,0.01 ,0.02mole] of  $Mg(NO_3)_2 \cdot 6H_2O$  respectively.

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The change in the lattice parameters of Mg–HA clearly demonstrated that Mg ions were structurally incorporated into HA crystals; they did not just cover the surface of the crystals.

The crystallinity of the samples decreased as the Magnesium ions concentrations increase as shown in table (2). This is may be due to the substitution of Ca ions by the Mg ions which cause deformation in the lattice structure of the HA crystal. The crystallinity evaluated by the following equation [17]:-

$$X_c = 1 - \frac{V_{112/300}}{I_{300}}$$

**Table2.** The degree of crystallinity of prepared HA(S1) and Mg loaded hydroxyl apatite (S2,S3andS4) of concentrations [0.004,0.01 and 0.02mole] of  $Mg(NO_3)_2 \cdot 6H_2O$  respectively.

Materials	Crystallinity ( $X_c$ ) (%)
S1	75
S2	50
S3	40
S4	35.71

The crystallite size is decreased by doping small amount of  $Mg^{2+}$  ions and increased with increasing the amount of  $Mg^{2+}$  ions due to increases of the lattice disorder as a result of the presence of Magnesium ions, as in table (3).

**Table3.** The crystallite size of prepared HA(S1) and Mg loaded hydroxyl apatite (S2,S3andS4) of concentrations [0.004,0.01,0.02mole] of  $Mg(no_3)_2 \cdot 6H_2O$  respectively.

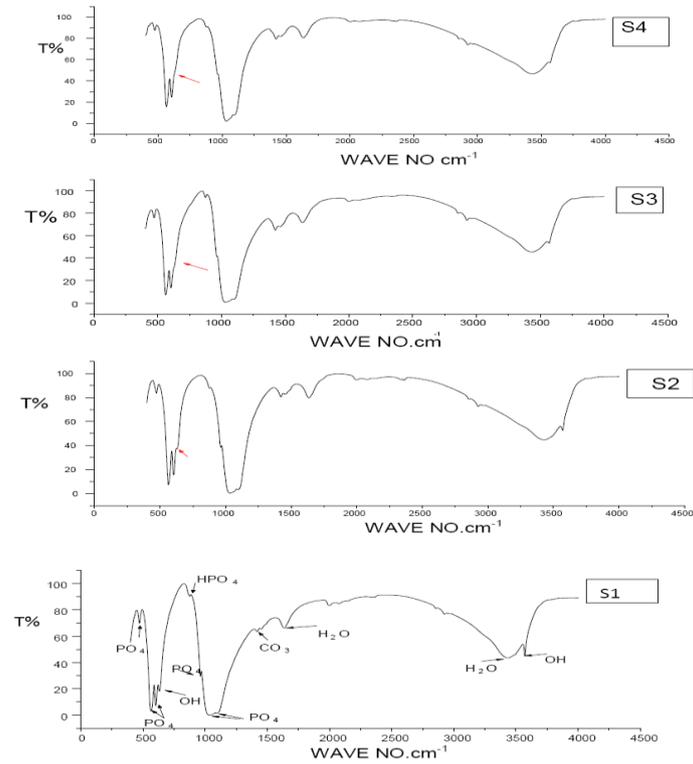
	Crystallite size, $L_{112}$ (nm) by Scherrer's equation
S1	35
S2	28.4
S3	27.4
S4	28.7

$Mg^{+2}$  doping reduced the intensity of the peaks and adsorption bands which induced the formation of calcium ( $Ca^{+2}$ ) deficient HA. The formation of calcium deficient HA showed that  $Mg^{+2}$  was substituted into HA structure by replacing the vacancy in  $Ca^{+2}$  position[18].

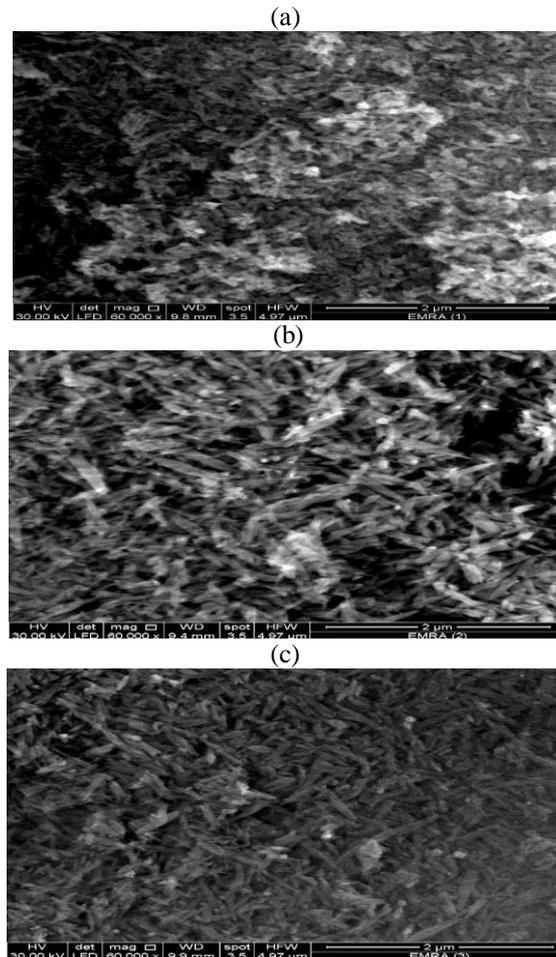
FTIR pattern for the prepared samples as in fig. (2) shows the following bands, The two bands at 3571 and 633.50  $cm^{-1}$  are assigned to the stretching and bending modes of OH groups, respectively. The presence of  $(PO_4)^{-3}$  is attested by 1) the P-O stretching modes appearing at 1093, 1031  $cm^{-1}$  ( $\nu_3$ ) and 962  $cm^{-1}$  ( $\nu_1$ ); 2) the O-P-O bending modes observed at 603, 565  $cm^{-1}$  ( $\nu_4$ ) and 472  $cm^{-1}$  ( $\nu_2$ ) [19]. Besides those typical bands of CaHAp, two bands at 1635  $cm^{-1}$  and around 3435  $cm^{-1}$  are due to adsorbed molecular water, whereas two small peaks at 1418 and 1450  $cm^{-1}$  indicate the presence of carbonate group. In addition, the shoulder at 875  $cm^{-1}$  is assigned to  $(HPO_4)_2$  groups formed upon reaction of some surface  $(PO_4)^{3-}$  with water. The presence of Mg in the samples leads to the decrease of the intensity of the band at 3571  $cm^{-1}$  and 633.50  $cm^{-1}$ , assigned to the vibrations of structural OH groups located in apatite tunnels. This intensity decrease may be ascribed to the dehydroxylation which might accompany the formation procedure.

Scanning electron microscopy demonstrated that HAp sample is consists of plate like surface morphology showing nonuniform particle distribution as in fig.(3.a), a morphology typical to HA powders synthesized via this route. [20,21]. The morphology of The Mg-HA particles is clearly rod shape. fig (3.b,c). EDX analysis as in fig. (4) shows that the substitution of the Mg ions increases as the doping concentrations of Mg increased and this indicated that Ca ions substituted by Mg .

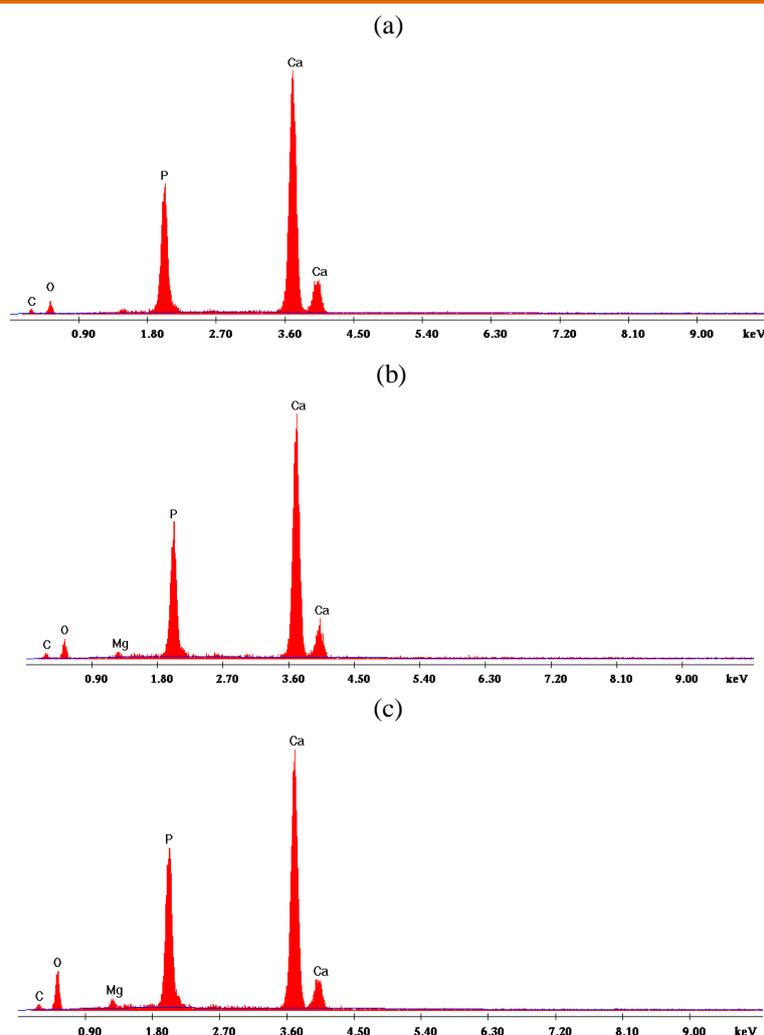
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**Fig2.** FTIR pattern of prepared HA(S1) and Mg loaded hydroxyl apatite (S2 , S3andS4) of concentrations [0.004,0.01,0.02 mole] of  $Mg(NO_3)_2 \cdot 6H_2O$  respectively.



**Fig3.** Shows SEM for the HA as in (a) and MHA of concentrations (0.01 & 0.02 ) mole of  $Mg(NO_3)_2 \cdot 6H_2O$  as in (b) & (c) respectively.



**Fig4.** Shows the SEM–EDX results of HA sample as in (a), and of MHA of concentrations ( 0.01 & 0.02 mole of  $Mg(NO_3)_2 \cdot 6H_2O$  as in (b) & (c) respectively.

## 5. CONCLUSION

- The wet precipitated method used to prepare pure hydroxyapatite and hydroxyapatite doped with different concentrations of magnesium.
- XRD, FTIR, SEM and EDX used to characterize the prepared samples as in figures [ 1,2,3 and 4 ].
- It was noticed that there is no peak of crystalline magnesium was detected in the diffractograms, the lattice parameters of samples show lattice parameters decrease upon magnesium addition, demonstrated that Mg ions were structurally incorporated into HA crystals; they did not just cover the surface of the crystals.
- The crystallinity of the samples decreased as the magnesium ion concentrations increase as in table (2).
- The morphology of the Mg-HA particles is clearly rod shape as shown in fig.(3). EDX analysis shows that the substitution of the Mg ions increases as the doping concentrations of Mg increased and this indicated that Ca ions substituted by Mg as shown in fig (4).
- Next research will use the MHA in medical application.

## REFERENCES

- [1] Jarcho M, Kay JF, Gumar KI, Doremus RH, Drobeck HP. (1977) "Tissue, cellular and subcellular events at a bone ceramic hydroxyapatite interface." *J.BiosciBioeng*; vol. 1: pp.79-92.
- [2] Landi E, Tampieri A, Mattioli-Belmonte M, Celotti G, Sandri M, Gigante A, (2006 )" Biomimetic Mg- and  $MgCO_3$ -substituted hydroxyapatites: synthesis characterization and in vitro behavior." *Journal of the European Ceramic Society*; vol.26: pp.2593-2601.

- [3] Rude RK, Gruber HE. (2004) "Magnesium deficiency and osteoporosis: animal and human observations." *Journal of Nutritional Biochemistry*;vol.15: pp.710-716.
- [4] Luoma H. (1988) " The role of magnesium in the aetiology and prevention of caries: some new findings and implications. Magnesium research." official organ of the International Society for the Development of Research on Magnesium;Vol.1: pp.223-230.
- [5] Cacciotti I, Bianco A, Lombardi M, Montanaro L (2009): "Mg-substituted hydroxyapatite nanopowders: Synthesis, thermal stability and sintering behavior." *JOURNAL OF THE EUROPEAN CERAMIC SOCIETY*, vol. 29, pp. 2969-2978.
- [6] Adzila S, Murad M. C, Sopyan I. (20 12)"Doping metal into calcium phosphate phase for better performance of bone implant materials ". *Rec. Pat. Mater.Sci. Vol. 5*, pp. 18- 47.
- [7] LeGeros RZ. (1991 )"Calcium phosphates in oral biology and medicine." *Monographs in oral science*, vol. 15, pp.1-201.
- [8] Bertoni E, Bigi A, Cojazzi G, Gandolfi M, Panzavolta S, Roveri N.(1998) " Nanocrystals of magnesium and fluoride substituted hydroxyapatite." *J.InorgBiochem*;vol72 , pp.29-35.
- [9] Gomes S, Renaudin G, Jallot E, Nedelec JM. (2009) "Structural Characterization and Biological Fluid Interaction of Sol-Gel-Derived Mg-Substituted Biphasic Calcium Phosphate Ceramics." *ACS Applied Materials & Interfaces*; vol.1, pp.505-513.
- [10] Landi E, Logroscino G, Proietti L, Tampieri A, Sandri M, Sprio S. (2008)."Biomimetic Mg-substituted hydroxyapatite: from synthesis to in vivo behavior." *Journal of Materials Science: Materials in Medicine*;vol.19: pp.239-247.
- [11] Bouyer E, Gitzhofer F, Boulos I (2000) : "Morphological study of hydroxyapatite nanocrystal suspension." *J. Mater Sci Mater Med*, vol.11: pp. 523-531.
- [12] Hammond C. (2002 ): "The Basics of Crystallography and Diffraction ." New York: Oxford University Press;3rd Edi. Vol. 416.
- [13] Koutsopoulos S. (2002): "Synthesis and Characterization of Hydroxyapatite Crystals.": A Review Study on the Analytical Methods. *J. Biomed Mater Res*,vol. 62: pp. 600-612
- [14] Miyaji F., Kono Y., (2005) "Formation and structure of zinc substituted calcium hydroxyapatite." "*Materials Research Bulletin* vol. 40, pp. 209–220.
- [15] Samira M. Sallam, Khairi M. Tohami, Abdelsattar M. Sallam, Lotfi I. Abo Salem, Faten Adel Mohamed( 2012)"Synthesis and characterization of hydroxyapatite contain chromium". *Journal of Biophysical Chemistry*, Vol.3, pp. 278-282.
- [16] Marcia S. Sader,KanthiLewis , Gloria A. Soares, Racquel Z. LeGeros(2013) . "Simultaneous incorporation of magnesium and carbonate in apatite: effect on physico-chemical properties." *Carlos Mat. Res. vol.16 .pp.779-784.*
- [17] Landi E, Tampieri A, (2000) "Densification behavior and mechanism of synthetic hydroxyapatite." *Journal of European ceramic society*, Vol.20, pp.2377-2387.
- [18] Adzila S, Ramesh S, Sopyan I, Tan C.Y, Hamdi M and Teng W.D. (2013) "Mechanochemical Synthesis of Magnesium Doped Hydroxyapatite Powder Characterization." *Applied Mechanics and Materials. Vol. 372* pp 62-65.
- [19] Crosman, A, Gelbard, G, Poncelet, G and Parvulescu, V.I.(2004)" Oxidative dehydrogenation of propane over chromium-loaded calcium-hydroxyapatite." *Applied Catalysis A: General*,vol23, pp. 264.
- [20] Mishra V.K, Srivastava S.K, Asthana B.P, Kumar D. (2012) "Structural and Spectroscopic Studies of Hydroxyapatite Nanorods Formed via Microwave-Assisted Synthesis Route." *J. Am. Ceram. Soc.*vol. 95, pp. 2709–2715.
- [21] Lak A, Mazloumi M, MohajeraniM.S,Zanganeh S, ShayeghM.R,Kajbafvala A., Arami H., Sadrnezhad S.K. (2008): "rapid formation of mono-dispersed hydroxyl apatite nano rods with narrow- size distribution via irradiation ." *J. Am. Ceram. Soc. Vol. 91*,pp. 3580–3584.